



## Effect of Abalone Hydrolysates Encapsulated by Double Emulsion on the Physicochemical and Sensorial Properties of Fresh Cheese

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### Abstract

The intake of dietary salt through food now exceeds current nutritional recommendations and is thought to have negative effects on human health, such as the increasing prevalence of hypertension. This study was performed to investigate whether  $W_1/O/W_2$  double emulsions can be used to enhance the saltiness of cheese without increasing the salt content ( $W_1$  is distilled water or 1% abalone hydrolysate, and  $W_2$  is 1% NaCl or 1% abalone hydrolysate + 1% NaCl solution). We also investigated the effect of adding abalone hydrolysate to the double emulsion as a saltiness enhancer. The cheeses were physico-chemically evaluated to determine curd yield, pH value, moisture content, color, texture, salt release rate, and sensory properties. No significant differences were observed in curd yield, pH value, moisture content, lightness, or redness between the cheeses made with and without the double emulsion. However, in the evaluation of salt release rate, fresh cheese made with double emulsion ( $W_1$  = distilled water,  $W_2$  = 1% NaCl + 1% abalone hydrolysate) was detected earlier than the control or the other treatments. In the sensory evaluation, fresh cheese made with the double emulsion showed higher scores for saltiness and overall preference than the control or the other treatments. We concluded that abalone hydrolysate encapsulated in a double emulsion ( $W_1$  is water and  $W_2$  is abalone hydrolysate and NaCl solution) could enhance the saltiness of fresh cheese while maintaining the same salt concentration, without altering its physical properties.

**Keywords** double emulsion, fresh cheese, salt enhancer, abalone hydrolysate

### Introduction

In many industrialized countries, the dietary intake of sodium currently exceeds nutritional recommendations, and many studies have reported a correlation between dietary salt intake, and the prevalence of hypertension (Guàrdia *et al.*, 2006). As consumer interest in healthy food has grown, salt quantity has become a major issue for the food industry (Evert-Arriagada *et al.*, 2012). According to Kotchen and McCarron (1998), a healthy daily sodium intake is < 2400 mg.

Salt is essential ingredient in most dairy products including cheese (Cecchinato *et al.*, 2015). There are several important role such as a source of sodium, a flavor enhancer, and a preservative to control enzyme activity and microbial growth, and develops flavor and aroma during cheese ripening (Guinee, 2004; Guinee *et al.*, 2004). Along with calcium level and pH, the salt content strongly influences cheese functionality, both directly and indirectly. A higher salt content increases the hardness and decreases the cohesiveness of cheese (Cervantes *et al.*, 1983).

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The salt content also affects the structure of cheese, because salt enhances solubilization of caseins, causing the casein matrix to swell and form a hydrated gel (Guinee, 2004; Guo and Kindstedt, 1995). Therefore, salt is essential in cheese, and the salt concentration affects the cheese quality (Floury *et al.*, 2009).

Salt enhancers and salt replacers are used to reduce the salt content of food without decreasing the quality (Lee *et al.*, 2016). Salt enhancers such as monosodium glutamate (MSG), amino acids, and yeast extract, improve the salty and savory taste of food (Famelart *et al.*, 1999). Salt replacers include sodium citrate, KCl, and calcium ascorbate (Lee *et al.*, 2016). In this study, abalone extract was used as a salt enhancer since it contained high concentration of lysine and glutamic acid which was main component of salt enhancer (Yoo and Chung, 2007). Besides that the abalone has various health benefits such as promotion of healthy eyes, alleviation of colds, improvement of blood circulation (Bansemer *et al.*, 2014).

Double emulsions consist of liquid droplets within larger liquid droplets, which are themselves dispersed in a secondary phase (O'Regan and Mulvihill, 2010). There are two main types of double emulsions: (1) water in oil in water ( $W_1/O/W_2$ ) emulsions, in which a W/O emulsion is dispersed in a secondary aqueous phase, (2) oil in water in oil ( $O_1/W/O_2$ ) emulsions, in which an O/W emulsion is dispersed in a secondary oil phase (Wolf *et al.*, 2009). Water in oil in water ( $W_1/O/W_2$ ) emulsions are commonly used and studied in the food industry because hydrophilic emulsifiers offer more options used to stabilize the continuous phase compared to lipophilic emulsifiers (O'Regan and Mulvihill, 2010). Recently, double emulsions have been applied for the purpose of masking off-flavor, reducing fat content, preventing lipid oxidation, enhancing flavors as well as improving organoleptic properties (O'Regan and Mulvihill, 2010). It is likely that a water-soluble ingredient such as salt can be encapsulated by double emulsion by which the stability of the emulsion is improved. However, there were few study on the effect of sol-

ute concentration in continuous phase to enhance the saltiness when it is applied to a food formulation. Therefore, this study evaluated the effects of  $W_1/O/W_2$  double emulsion containing abalone hydrolysate on the quality characteristics and sensorial properties of fresh cheese.

So, the purpose of this study was to evaluate the effect of use of a  $W_1/O/W_2$  double emulsion containing abalone hydrolysate in production of cheese.

## Materials and Methods

### Materials

Commercial pasteurized milk was purchased from the Seoul Dairy Co-op (Korea). Liquid animal rennet, edible citric acid, and calcium chloride used for the production of cheese curd were obtained from Cheesemaking Supply Co. (USA), Jungbunzlauer (Switzerland), and Daejung Chemical Co. (Korea). To prepare the double emulsions, whey protein isolate (WPI) (Milk Specialties Global, USA), edible medium-chain triglyceride oil (MCT oil) (Bolak Co., Ltd., Korea), polyglycerol polyricinoleate (PGPR) (Il-sin Co., Ltd., Korea), k-carrageenan (MSC Co., Ltd., Korea), polyoxyethylene sorbitan monooleate (Tween® 80) (Shamchun Pure Chemical Co., Ltd., Korea), abalone hydrolysate (Woosung F&B Co., Ltd, Korea), and salt (Chungjungone, Deasang Co., Korea) were used. To observe the double emulsion, methylene blue trihydrate (Samchun pure chemicals Co., Ltd, Korea) and Oil Red O (Sigma-Aldrich, USA) was used as a dye.

### Preparation of $W_1/O/W_2$ double emulsion

The double emulsion was prepared by modified method of Gu *et al.* (2005). Table 1 shows the components of the double emulsion used for cheese production. The inner aqueous phase ( $W_1$ ) was prepared by hydrating WPI (2%, w/v) in water. The oil phase (O) was MCT oil containing 8%, w/v, of PGPR as a surfactant for  $W_1/O$  emulsion. The external water phase ( $W_2$ ) was prepared by dissolving k-carrageenan 1% (w/v) and Tween 80 (polyoxyethylene

**Table 1. Components used for preparation of cheese with double emulsions**

		Addition of double emulsion	
		$W_1$ (Internal water phase)	$W_2$ (Internal water phase)
Control	NaCl solution		
A	Abalone hydrolysate		
B		Water	NaCl solution
C		Abalone hydrolysate	NaCl solution
D		Water	NaCl solution + Abalone hydrolysate

sorbitan monooleate) 2% (w/v) in 1% NaCl solution without or with abalone hydrolysate. The water in oil ( $W_1/O$ ) emulsion was created by mixing the inner aqueous phase ( $W_1$ ) and the oil phase (O) at a 1:4 ratio, followed by homogenization at 10,000 rpm for 2 min using a high-speed homogenizer (IKAT25, Digital ULTRA-TURRAX<sup>®</sup>, Germany). The  $W_1/O$  emulsion was dispersed into the outer aqueous phase at a ratio of 1:4, and the mixture was homogenized at 5,000 rpm for 2 min using a high-speed homogenizer (IKAT25, Digital ULTRA-TURRAX<sup>®</sup>, Germany).

### Preparation of fresh cheese

The fresh cheese was prepared using a modified version of the method described by Lobato-Calleros *et al.* (2008) and Lobato-Calleros *et al.* (2007). Milk was stored at 5°C until use. Before mixing with the double emulsion, 500 g of milk was heated at 37°C in a shaking water bath (BF-30SB, Biofree, Korea) for 30 min, 60 rpm. The heated milk was added to 2 g of edible citric acid (0.4%, w/w), 0.5 mL of a 40% calcium chloride solution (0.1%, w/w), and liquid animal rennet (0.1%, v/w) for production of cheese curd. For firm coagulation, the water bath temperature was raised to 50°C for 50 min. After 50 min, 5 mL of 1% NaCl solution or one of four different double emulsions ( $W_1/O/W_2$ ) was added. After a coagulation time of approximately 30 min, the curd was cut into 1 cm<sup>3</sup> cubes, and then approximately 80% of the whey was drained. The curds were transferred to a cotton cloth in a sieve, stored at room temperature for 2 h, and the whey was removed. Then, the curds were placed in a plastic barrel, and stored in a refrigerator (4°C) overnight.

### Microscopic observation of the double emulsion droplets

Freshly prepared double emulsions were observed at 400× magnification under an optical microscope (CX31, Olympus, Japan) coupled with a CCD camera (3.0 M, Olympus, Japan). To distinguish the internal water phase in the oil droplets, the water ( $W_1$ ) in the  $W_1/O/W_2$  double emulsions was stained with 0.5% methylene blue solution hydrated with distilled water, or the MCT oil was stained with Oil Red O. The 0.5% methylene blue solution was added to 1% of internal water phase volume, and Oil Red O was added to 1% of MCT oil volume. In order to distinguish the shape of double emulsion, the methylene blue and red oil O was used to dye internal water and oil phase, respectively, before homogenization.

### Yield of fresh cheese

To determine the yield of curd production, the weight of the milk used to make the cheese and that of the final cheese with all whey removed were measured. The yield was expressed as a percentage, as shown in the following equation.

$$\text{Yield of curd production (\%)} = \frac{\text{Weight of curd (g)}}{\text{Weight of milk (g)}} \times 100$$

### pH value of fresh cheese

Studies have shown that pH value affects cheese quality (Marchesseau *et al.*, 1997). A 3 g sample of fresh cheese and 27 mL of distilled water were ground in a household blender (CNHR 26, Bosch, Slovenia) for 1 min. The pH values of samples were determined using a pH meter (Orion 3-STAR, Thermo Scientific, USA).

### Moisture content of fresh cheese

The moisture content was determined in triplicate using the method described by the AOAC (1990). Curd was dried by heating to extract the moisture. Two-gram samples of curd were put in weighing bottles and dried in an oven (WOF-105, Daihan Scientific, Korea) for 24 hours. Drying was continued until the samples showed no change in weight. The water content was determined by comparing the weight of the sample before and after drying.

### Color

Samples of fresh cheese were placed in 3-cm petri dishes and the color was determined using a color reader (CR-10, Konica Minolta Sensing Inc., Japan) calibrated with a white standard plate ( $L^* = 94.51$ ,  $a^* = -0.66$ ,  $b^* = 3.31$ ). The CIE  $L^*$ ,  $a^*$ , and  $b^*$  values were designated as indicators of lightness, redness, and yellowness, respectively. The total color difference ( $\Delta E$ ) was numerically calculated by comparing the color of the controls and treated samples using the following equation.

$$\Delta E = \sqrt{(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2}$$

$L_1^*$ ,  $a_1^*$ ,  $b_1^*$  = lightness, redness, and yellowness of control

$L_2^*$ ,  $a_2^*$ ,  $b_2^*$  = lightness, redness, and yellowness of treatment

### Texture profile analysis (TPA)

TPA was performed using a texture analyzer (CT3,

Brookfield Engineering Labs Inc., USA) equipped with a TA39 probe and a TA-RT-KI fixture. Before measuring, 20 g of sample was placed in a cylindrical vial (22 × 50 mm; width × height). Samples were penetrated to 50% of their original height at a test speed of 2 mm/s and a trigger load of 5 g. The analysis was carried out at least 6 times. Values for hardness ( $g_f$ ), adhesiveness (mJ), cohesiveness, springiness (mm), and chewiness (mJ) were determined.

#### Salt release rate

The salt content of the cheese samples was determined using a 2 g sample homogenized in 100 mL distilled water using a magnetic stirrer (MSH-20D, Daihan Scientific, Korea) at 300 rpm for 600 s. The salt content was determined with a salimeter (8603, Mettler Toledo GmbH, Switzerland).

#### Sensory evaluation

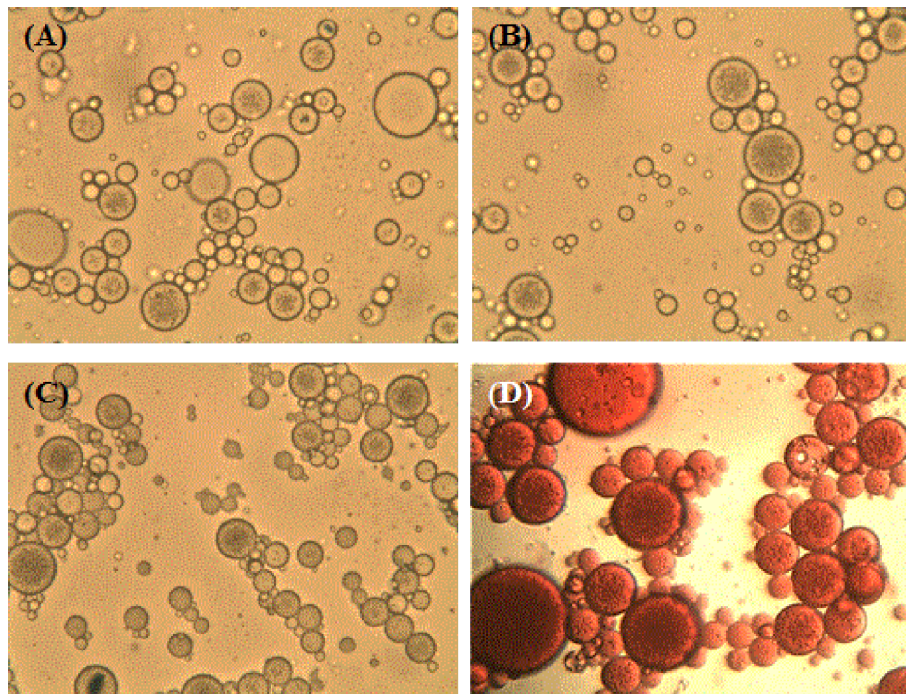
After storage overnight in a refrigerator (4°C), a 1 g sample was cut from each of the four cheeses. Samples were coded with three-digit random numbers, and randomly assigned to 8 panelists trained to test flavor, salty

intensity, oiliness, and overall preference. The panelists' preference for each cheese was ranked on a hedonic scale (5 = like extremely, 4 = like moderately, 3 = moderately, 2 = dislike moderately, 1 = dislike extremely).

## Results and Discussion

### Microscopic observation of the double emulsion droplets

Fig. 1 clearly shows that the internal water phase was entrapped in the oil droplets in all samples. The image clearly shows the fine dispersion of the internal aqueous-phase droplets ( $W_1$ ) inside the large oil droplets that were dispersed in the outer aqueous phase ( $W_2$ ), confirming the formation of double emulsions. The double emulsions are classified by three types based on the size and number of internal aqueous phase droplets (Florence and Whiehill, 1981). Type A double emulsion is consisted of only one large internal droplet inside the oil phase droplet. For type B double emulsion, the internal aqueous phase is composed of several small droplets. Alternately, type C double emulsion has a large number of fine internal droplets. In our emulsion, the type C was dominant and many



**Fig. 1. Optical microscopy images of the water in oil in water (W1/O/W2) emulsions at 400× magnification.** (A): internal blank water phase stained by methylene blue in an oil droplet dispersed in an external water phase containing NaCl (1% NaCl in the external phase), (B): internal water phase stained by methylene blue containing abalone hydrolysate as a salt enhancer in an oil phase dispersed in an external water phase containing NaCl (1% NaCl in the external phase), (C): internal water phase stained by methylene blue in an oil droplet dispersed in an external water phase containing abalone hydrolysate and NaCl (1% NaCl in external phase).

**Table 2. Properties of curd prepared with addition of four different types of double emulsions**

	Yield (%)	pH	Moisture (%)
Control	24.10	5.04 ± 0.07 <sup>a</sup>	57.47 ± 1.06 <sup>a</sup>
A	20.00	5.07 ± 0.07 <sup>a</sup>	56.31 ± 1.34 <sup>a</sup>
B	20.90	5.16 ± 0.11 <sup>a</sup>	56.19 ± 1.51 <sup>a</sup>
C	23.00	5.02 ± 0.05 <sup>a</sup>	56.22 ± 1.16 <sup>a</sup>
D	22.50	5.02 ± 0.05 <sup>a</sup>	57.58 ± 1.93 <sup>a</sup>

Control: 1% NaCl, A: 1% abalone hydrolysate, B: internal blank water phase in oil droplet dispersed in external water phase containing NaCl (1% NaCl in external phase), C: internal water phase containing abalone hydrolysate as a salt enhancer in oil phase dispersed in external water phase containing NaCl (1% NaCl in external phase), D: internal water phase in oil droplet dispersed in external water phase containing abalone hydrolysate and NaCl (1% NaCl in external phase).

<sup>a</sup>Means with same superscripts in each column are significantly not different ( $p > 0.05$ ).

small droplets are encapsulated in the relatively larger droplets. This means that the emulsion is dominated by large droplets. Type C shows slower release of active substances than A or B (Iqbal *et al.*, 2017).

#### pH, yield of curd and moisture contents

The pH values of the fresh cheeses are given in Table 2. The pH of cheese did not change significantly with the type of emulsion. Oloffs *et al.* (1992) observed that desirable milk coagulation is directly correlated with the pH value of milk. The pH values of all samples ranged from 5.02 to 5.16, and the pH of samples treated with salt enhancers or double emulsions showed no significant differences as compared to the control ( $p > 0.05$ ).

Since the same amount of citric acid was added to all samples for pH control, the pH value did not differ significantly with double emulsion type. Table 2 presents the curd yield of the cheeses prepared using the various double emulsions. The curd yield value was <25% for all samples. The curd yield of samples C and D was higher than that of samples A and B indicating that addition of double emulsions containing abalone hydrolysate produces a higher curd yield than other double emulsions. In general, curd production is related to milk protein. Cow's milk contains 3-5% protein, 20% of which is whey protein and 80% of which is casein (Patel *et al.*, 2008). Ferragina *et al.* (2013) and Sturaro *et al.* (2015) reported that the mean curd yield was 15.04% and 20.86%, respectively. Lobato-Calleros *et al.* (2008) reported that there was a significant difference in curd yield when double emulsions containing gum arabic, carboxymethylcellulose, or low-methoxyl pectin were added to cheese.

Moisture contents of the fresh cheeses are shown in Table 2. The moisture contents of the fresh cheese ranged from 56.19% to 57.58% and there was no significant difference among samples ( $p > 0.05$ ). In contrast, Giroux *et al.* (2013) reported that the moisture content of cheese made with double emulsion was higher than controls. Cervantes *et al.* (1983) reported that increasing the salt content did not decrease the moisture content of cheese.

#### Color

The averages of the  $L^*$ ,  $a^*$ , and  $b^*$  values of the cheese samples, and the  $\Delta E$  values are shown in Table 3. For the control group, the average values for  $L^*$ ,  $a^*$ , and  $b^*$  were 88.31, -1.84, and 6.71, respectively. Sample A differed significantly between the treatment groups and the control group ( $p < 0.05$ ), owing to the addition of abalone hydrolysate which is a dark brown color. The  $b^*$  value of sample B was lower than the others owing to the absence of abalone extract in the double emulsion. The color of food is an important sensory attribute that can change consumers' flavor perception (Young *et al.*, 2004). In the case of cheese, it is important to recognize that the color of the cheese could affect the attitude of the consumer (Singh, 2006). Evert-Arriagada *et al.* (2012) and Sant'Ana *et al.* (2013) studied the  $L^*$ ,  $a^*$ , and  $b^*$  values of fresh cheese were 93.54, -3.16, 16.34, and 95.32, -60.0, 9.45, respectively.

Kwak *et al.* (2010) observed that addition of anchovy soluble protein extract causes significant changes in the color value of sausage, and Famelart *et al.* (1999) reported that when hydrolysate is added to cheese instead of salt, the physicochemical properties of the cheese are changed.

**Table 3. Color of curd prepared with addition of four different types of double emulsions**

	$L^*$	$a^*$	$b^*$
Control	88.01 ± 0.33 <sup>a</sup>	-1.84 ± 0.03 <sup>a</sup>	6.71 ± 0.21 <sup>ab</sup>
A	87.78 ± 0.10 <sup>a</sup>	-1.86 ± 0.08 <sup>a</sup>	6.57 ± 0.08 <sup>b</sup>
B	87.74 ± 1.03 <sup>a</sup>	-1.86 ± 0.09 <sup>a</sup>	7.07 ± 0.41 <sup>a</sup>
C	87.87 ± 0.12 <sup>a</sup>	-1.87 ± 0.02 <sup>a</sup>	6.64 ± 0.05 <sup>ab</sup>
D	87.27 ± 0.22 <sup>a</sup>	-1.82 ± 0.04 <sup>a</sup>	6.72 ± 0.04 <sup>ab</sup>

Control: 1% NaCl, A: 1% abalone hydrolysate, B: internal blank water phase in oil droplet dispersed in external water phase containing NaCl (1% NaCl in external phase), C: internal water phase containing abalone hydrolysate as a salt enhancer in oil phase dispersed in external water phase containing NaCl (1% NaCl in external phase), D: internal water phase in oil droplet dispersed in external water phase containing abalone hydrolysate and NaCl (1% NaCl in external phase).

<sup>a,b</sup>Means with different superscripts within the column are significantly different ( $p < 0.05$ ).

**Table 4. Texture properties of curd prepared with addition of four different types of double emulsions**

	Hardness (g)	Adhesiveness (mJ)	Cohesiveness	Springiness (mm)	Chewiness (mJ)
Control	77.33 ± 8.30 <sup>a</sup>	3.01 ± 0.84 <sup>a</sup>	0.68 ± 0.07 <sup>ab</sup>	12.75 ± 0.64 <sup>a</sup>	5.72 ± 0.76 <sup>a</sup>
A	54.10 ± 4.21 <sup>b</sup>	2.17 ± 0.36 <sup>b</sup>	0.63 ± 0.08 <sup>b</sup>	12.85 ± 0.67 <sup>a</sup>	4.07 ± 0.78 <sup>b</sup>
B	50.50 ± 4.92 <sup>b</sup>	2.33 ± 0.25 <sup>b</sup>	0.71 ± 0.05 <sup>a</sup>	13.15 ± 0.32 <sup>a</sup>	4.31 ± 0.43 <sup>b</sup>
C	56.56 ± 4.88 <sup>b</sup>	2.27 ± 0.59 <sup>b</sup>	0.69 ± 0.07 <sup>a</sup>	13.09 ± 0.23 <sup>a</sup>	4.68 ± 1.18 <sup>b</sup>
D	56.57 ± 6.00 <sup>b</sup>	2.05 ± 0.51 <sup>b</sup>	0.58 ± 0.06 <sup>c</sup>	12.58 ± 0.76 <sup>a</sup>	3.88 ± 0.79 <sup>b</sup>

Control: 1% NaCl, A: 1% abalone hydrolysate, B: internal blank water phase in oil droplet dispersed in external water phase containing NaCl (1% NaCl in external phase), C: internal water phase containing abalone hydrolysate as a salt enhancer in oil phase dispersed in external water phase containing NaCl (1% NaCl in external phase), D: internal water phase in oil droplet dispersed in external water phase containing abalone hydrolysate and NaCl (1% NaCl in external phase).

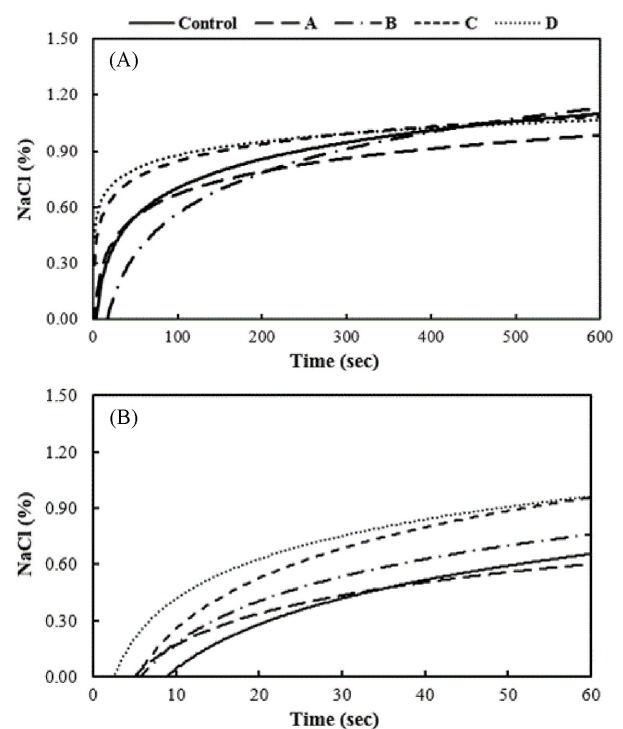
<sup>a-c</sup>Means with different superscripts in each column are significantly different ( $p < 0.05$ ).

### Texture profile analysis (TPA)

The texture properties of the fresh cheese samples are presented in Table 4. Kaya (2002) observed that salt concentration and hardness are associated in cheese. However, here the control showed higher hardness and adhesiveness values than cheese treated with the double emulsions, in spite their having the same salt concentration ( $p < 0.05$ ). We hypothesized that the MCT oil in the double emulsion increased the tenderness of the cheese. Serrano *et al.* (2005) observed that adhesiveness and oiliness are associated with the presence of oil in cheese. Significant differences in cohesiveness were observed between the control and other cheeses ( $p < 0.05$ ). Lee *et al.* (2016) showed that addition of hydrolysate, such as abalone hydrolysate into cooked noodles did not significantly change the cohesiveness or chewiness, in contrast to the results reported here.

### Salt release rate

In order to compare the results of sensory test at next section, the concentration of salt was measured over time by using instrument. Fig. 2 showed salt release rate over time, which indicates the degree of salt concentration detected. Fig. 2A indicated that the value became constant after 600 s in order to observe the releasing ending point. Fig. 2B showed the magnified from the Fig. 2A from 0 s to 60 s for the food chewing time in mouth, generally. As shown in Fig. 2, relative to the values of other samples, the salt concentration of the cheese made with the double emulsion containing the salt in the inner or external phase (sample C, D) or without abalone hydrolysate (sample B) was highest at the beginning, indicating a high NaCl release rate. This indicates that the external water phase containing NaCl can be quickly released during stirring due to localization of the NaCl in the outer phase. Comparatively, the NaCl release rate in cheese made without the double emulsion was slower than with



**Fig. 2. Salt release rate of curd prepared using double emulsions.** Control: 1% NaCl, A: 1% abalone hydrolysate, B: internal blank water phase in an oil droplet dispersed in an external water phase containing NaCl (1% NaCl in the external phase), C: internal water phase containing abalone hydrolysate as a salt enhancer in an oil phase dispersed in an external water phase containing NaCl (1% NaCl in the external phase), D: internal water phase in an oil droplet dispersed in an external water phase containing abalone hydrolysate and NaCl (1% NaCl in the external phase), (A): salt release rate during 600 s, (B): salt release rate during 60 s.

the double emulsion. At 30 s, the NaCl concentrations of sample D and the control were 0.62% and 0.34% (Fig. 2B). These results indicate that sample D had a saltier taste than the other treatments, in accordance with the results of the saltiness rankings from the sensory test (Table 5).

**Table 5. Sensory evaluation of curd prepared with addition of four different types of double emulsions**

	Salty intensity	Oiliness	Overall preference
Control	2.13 ± 1.64 <sup>b</sup>	3.80 ± 1.64 <sup>b</sup>	2.33 ± 1.23 <sup>c</sup>
A	2.25 ± 1.28 <sup>b</sup>	1.80 ± 1.30 <sup>b</sup>	2.53 ± 1.36 <sup>bc</sup>
B	3.38 ± 1.30 <sup>a</sup>	2.80 ± 0.84 <sup>ab</sup>	3.07 ± 1.49 <sup>a</sup>
C	3.38 ± 1.19 <sup>a</sup>	3.40 ± 1.52 <sup>ab</sup>	3.27 ± 1.33 <sup>ab</sup>
D	3.88 ± 1.13 <sup>a</sup>	3.20 ± 1.48 <sup>a</sup>	3.80 ± 1.37 <sup>a</sup>

Control: 1% NaCl, A: 1% abalone hydrolysate, B: internal blank water phase in oil droplet dispersed in external water phase containing NaCl (1% NaCl in external phase), C: internal water phase containing abalone hydrolysate as a salt enhancer in oil phase dispersed in external water phase containing NaCl (1% NaCl in external phase), D: internal water phase in oil droplet dispersed in external water phase containing abalone hydrolysate and NaCl (1% NaCl in external phase).

<sup>a-c</sup>Means with different superscripts in each column are significantly different ( $p < 0.05$ ).

In general, the diffusion rate of electrolytes such as Na cations is driven by concentration gradients, due to differences in surfactants, electrolytes, osmotic regulators, and encapsulated compounds (Bonnet *et al.*, 2010). In our system, the shear force applied during stirring can break up the cheese, which can induce release of the NaCl in the double emulsion to the external water phase. For approximately 10 min, the concentration of NaCl remained constant at 1.0%, except in sample B. In the samples made with the double emulsion containing an internal water phase, the NaCl concentration of the dispersed phase did not decrease over time compared to the control.

### Sensory evaluation

The results of the sensory test are shown in Table 5. The average values for salt intensity, oiliness, and overall preference in the control group were 2.13, 3.80, and 2.33, respectively. For the treatment group, except sample A, the average values of salt intensity, oiliness, and overall preference were 3.88, 2.80-3.20, and 3.07-3.80, respectively. The salt intensity and overall preference for the cheeses prepared with the double emulsions were significantly higher than those of the control or sample B ( $p < 0.05$ ). In particular, sample D showed the highest scores for salt intensity and overall preference. It was considered that the abalone hydrolysates can play a role as a salt enhancer in the  $W_2$  phase of the double emulsions in cheese unlike the other samples, therefore it was affected to the value of overall preference. Moreover, since the NaCl was quickly released from the external phase of the cheese, the saltiness of the cheese could be rapidly detected on the mouth,

when the panel chewed the fresh cheese piece (Fig. 2). Generally, saltiness is associated with overall preference in food, and people tend to prefer food with a higher salt content (Matsuzuki *et al.*, 2008). For oiliness values, the cheese made with the double emulsions differed significantly from the cheese made without the emulsions, perhaps due to the MCT oil in the emulsions. As mentioned above, this result may be associated with texture, shown in Table 2. Therefore, we propose that application of the double emulsion of cheese could promote the saltiness of cheese, which may lead to reduce of the salt in cheese production. Thus, we consider that double emulsions containing a salt enhancer can be used in preparation of cheese with a reduced salt concentration but the same taste.

### Conclusion

The effect of double emulsions containing abalone hydrolysate on preparation of fresh cheese was analyzed for enhancing saltiness while maintaining the same salt concentration. No significant differences were observed in curd yield, pH value, moisture content, color, or texture between fresh cheeses made with and without the double emulsion. Based on salt release rate and sensory evaluation, fresh cheese made with a double emulsion containing NaCl and abalone hydrolysate in the external water phase had a saltier taste than the other cheeses. Further reduction of the NaCl concentration for production of low-salt cheese will be investigated in more detail in future research.

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